under fierce competition by commensal strains of *E. coli*.

These findings suggest that EHEC may have evolved mechanisms to avoid expending energy to transport and utilize a nutrient that is actively being used by other microbes, possibly giving EHEC a competitive advantage over the microbiota. The *fusKR* genes, while present in the EHEC O157:H7 serotype, are notably absent in all other published genomes of *E. coli*. It remains to be seen whether repression of fucose utilization by the FusKR response regulator confers a growth advantage upon EHEC because it can utilize alternative unique carbon sources while commensal strains of *E. coli* cannot, but it would be telling if transfer of the cognate FusKR pair to commensal strains of *E. coli* conferred a growth disadvantage to them in the host. This would raise the mirror question: if EHEC uses the FusKR two-component system to abstain from a carbon source that other microbiota members use while in the gastrointestinal tract, has EHEC gained specific mechanisms to utilize unique carbon sources that do not exist in commensal microbiota members?

The repression of virulence gene expression by a member of the microbiota when EHEC is in the presence of mucus raises many unanswered questions. Would an individual with an unbalanced microbiota scant in *Bacteroidetes* be more susceptible or less susceptible to EHEC disease? Do the varying concentrations of mucus and fucose present throughout the entire gastrointestinal tract have a direct influence upon the colonization site of EHEC within the large intestine? Will high fucose concentrations encountered by shedding EHEC cause the pathogen to conserve its energy by repressing virulence gene expression and result in a pathogen that is more or less fit for transmission? Future research will no doubt shed light on these issues.

References

1. Kaper, J.B., Nataro, J.P., and Mobley, H.L. (2004). Pathogenic Escherichia coli. Nat. Rev. Microbiol. *2*, 123–140.
2. Pacheco, A.R., Curtis, M.M., Ritchie, J.M., Munera, D., Waldor, M.K., Moreira, C.G., and Sperandio, V. (2012). Fucose sensing regulates bacterial intestinal colonization. Nature *492*, 113–117.
3. Njoroge, J.W., Nguyen, Y., Curtis, M.M., Moreira, C.G., and Sperandio, V. (2012). Virulence meets metabolism: Cra and KdpE gene regulation in enterohemorrhagic Escherichia coli. MBio. *3*, e00280–e00312.
4. Reading, N.C., Torres, A.G., Kendall, M.M., Hughes, D.T., Yamamoto, K., and Sperandio, V. (2007). A novel two-component signaling system that activates transcription of an enterohemorrhagic Escherichia coli effector involved in remodeling of host actin. J. Bacteriol. *189*, 2468–2476.
5. Xu, J., Bjursell, M.K., Himrod, J., Deng, S., Carmichael, L.K., Chiang, H.C., Hooper, L.V., and Gordon, J.I. (2003). A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science *299*, 2074–2076.
6. Robbe, C., Capon, C., Coddeville, B., and Michalski, J.-C. (2004). Structural diversity and specific distribution of O-glycans in normal human mucins along the intestinal tract. Biochem. J. *384*, 307–316.
7. Fabich, A.J., Jones, S.A., Chowdhury, F.Z., Cernosek, A., Anderson, A., Smalley, D., McHargue, J.W., Hightower, G.A., Smith, J.T., Autieri, S.M., *et al*. (2008). Comparison of carbon nutrition for pathogenic and commensal Escherichia coli strains in the mouse intestine. Infect. Immun. *76*, 1143–1152.

Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada.
E-mail: bfinlay@msl.ubc.ca

http://dx.doi.org/10.1016/j.cub.2012.12.027

Intestinal Regeneration: YAP—Tumor Suppressor and Oncoprotein?

The Hippo signaling pathway exerts a growth-suppressive effect by inhibitory phosphorylation of the oncogenic transcription co-activator Yki/YAP. A recent study paradoxically reports that genetic removal of YAP enhances intestinal stem cell expansion and regeneration.

Vivian S.W. Li[1,2,*] and Hans Clevers[1]

Recent studies have demonstrated important roles of the Hippo signaling pathway in organ-size control during development and tissue regeneration [1]. The highly conserved Hippo pathway was first identified in *Drosophila*: upstream signals (the identities of which are still under debate) activate a serine/threonine kinase cascade involving the kinases Hippo and Warts (Wts), which in turn leads to inhibitory phosphorylation of the downstream transcriptional co-activator Yorkie (Yki). Suppression of the Hippo pathway or overexpression of Yki results in massive tissue overgrowth in *Drosophila* [2], implying an oncogenic role for Yki. In vertebrates, the corresponding components are the kinases Mst and Lats, and the related transcriptional co-activators Yes-associated protein (YAP) and WW-domain-containing transcription regulator 1 (TAZ) [1]. Transgenic expression of YAP in the liver induces uniform expansion of liver mass, whereas prolonged expression of YAP results in tumorigenesis [3]. These combined data demonstrate that the Hippo pathway is a growth-suppressive pathway in both flies and mammals, and that the downstream effector Yki/YAP is a highly potent oncogene.

Several recent studies have addressed roles of the Hippo signaling pathway in self-renewal and repair of the intestinal epithelium. YAP protein is expressed at the bottom of crypts — the stem cell compartments of the intestinal epithelium [4]. Genetic inhibition of Hippo signaling results in hyperplasia of the gut epithelium in both *Drosophila* and mouse [5–7]. Consistently, activation of YAP in the intestine results in expansion of undifferentiated progenitor cells [4]. On the other hand, YAP appears to be dispensable in normal intestinal homeostasis since loss of intestinal YAP leads to no visible defects [6]. To study the potential regulatory role of the Hippo signaling pathway in intestinal regeneration, Cai *et al.* [6] performed a mouse study in the dextran sodium sulfate (DSS)-colitis model. YAP protein expression was elevated two days after

DSS-induced damage in regenerating colonic crypts. When YAP was deleted, the repair of DSS-induced intestinal damage was severely impaired. All of these data are consistent with a model in which YAP serves as a growth-promoting protein in the process of intestinal regeneration.

A new study, published recently in *Nature* [8], has now revealed an unexpected role of YAP in intestinal regeneration. Barry *et al.* [8] re-assessed the function of YAP in the mouse intestine. In contrast to the previously reported expansion of undifferentiated crypt progenitors upon ubiquitous expression of YAP, coincident with the nuclear accumulation of the Wnt effector β-catenin [4], Barry *et al.* [8] noted that the Wnt signaling pathway was inhibited and that intestinal stem cell markers were reduced upon overexpression of YAP protein specifically in the intestine. As a consequence, proliferative crypts were lost.

The major discrepancy between the two studies could possibly be explained by data collection at different time points. Doxycycline-induced YAP expression in the Cai *et al.* study [6] was maintained for up to 4.5 days and then followed by doxycycline withdrawal to analyze reversibility of the phenotype. In the latter study by Barry *et al.* [8], the degenerative crypt phenotype was first observed at day 7 and was accompanied by complete loss of Paneth cells, which are intermingled with stem cells at crypt bottoms. The data suggest that YAP overexpression inhibits the intestinal differentiation program, which eventually causes the Paneth cell loss. Of note, Paneth cells are a crucial component of the intestinal stem-cell niche [9]. Thus, YAP overexpression in the intestine temporarily suppresses differentiation in agreement with its 'generic' growth-promoting effect. Prolonged expression of YAP will eventually lead to loss of the niche and — paradoxically — thus cause progressive crypt degeneration.

Another set of surprising data from the study by Barry *et al.* [8] implies that YAP deletion can result in opposite phenotypes in different tissue-regenerative models. Barry *et al.* [8] confirmed the previous finding that YAP-deficient mice show an impaired regenerative response after DSS-induced tissue damage [6].

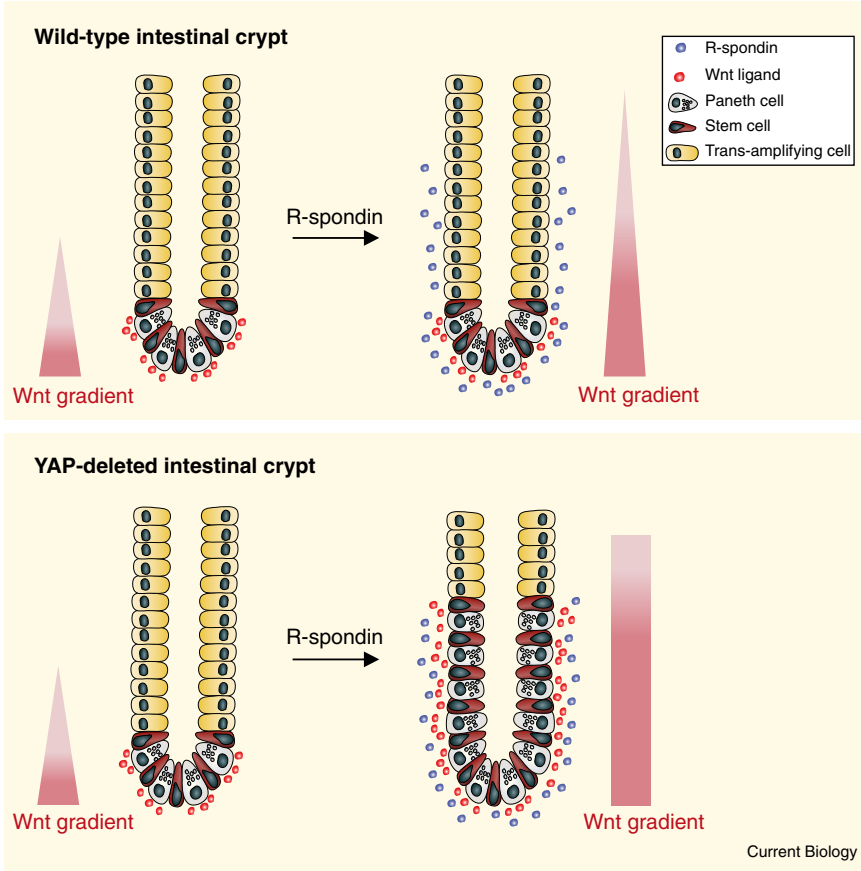

Figure 1. Model of Wnt activation in wild-type and YAP-deficient intestine upon R-spondin stimulation.

YAP deletion increases the number of Paneth cells after R-spondin induction, resulting in massive amplification of the Wnt signal.

Upon whole-body irradiation, however, YAP-deficient mice demonstrated crypt hyperplasia and overgrowth in both small intestine and colon [8]. The authors confirmed the growth-suppressive function of YAP by stimulation with the Wnt agonist R-spondin1 (Rspo1) [10,11], a potent growth factor for intestinal crypts [12]. Loss of YAP increased Wnt/Rspo1 hypersensitivity and induced massive hyperplasia, which was accompanied by upregulation of Wnt targets and intestinal stem cell markers [8]. Together, the data suggest opposite roles for YAP: in one role, YAP serves as an oncoprotein, promoting growth upon DSS-induced intestinal damage; and in the other role, YAP is a growth-repressive protein that restricts Wnt/Rspo1-induced intestinal stem-cell expansion and regeneration after irradiation-induced injury.

To better understand the distinct roles of YAP in tissue regeneration, it is important to distinguish the mechanistic differences between DSS- and irradiation-induced injury models. Oral administration of DSS disrupts mucin production by the intestinal mucosa and induces an inflammatory response, resulting in colitis-like lesions. It has been reported that DSS-induced intestinal epithelial regeneration requires activation of the Notch signaling pathway [13]. Treatment with DSS upregulates expression of the Notch target gene Hes1, whereas inhibition of Notch activation using a gamma-secretase inhibitor impairs DSS-induced intestinal regeneration [13]. Interestingly, YAP overexpression has been shown to induce the expansion of Hes1-expressing crypt progenitor cells, which in turn can be suppressed by gamma-secretase inhibitors [4]. Consistently, knockdown of YAP strongly decreases the abundance of the Notch intracellular domain and of Hes1 expression [7]. Taken together, DSS treatment may activate Notch

signaling in a YAP-dependent manner; loss of YAP would thus impair DSS-induced intestinal regeneration by inactivating the Notch pathway. The second injury model involves whole-body irradiation. Proliferative cells, such as crypt progenitors, are exquisitely sensitive to irradiation. In contrast to the DSS-induced regeneration, Barry *et al.* [8] observed upregulation of Wnt targets and increased numbers of Paneth cells in the YAP-deficient intestine after irradiation. The authors suggest that loss of YAP increases Wnt hypersensitivity, which is confirmed by the Rspo1 experiments. It is interesting to note that the number of Paneth cells is strikingly increased in the YAP knockout mice in both irradiated and Rspo1-stimulated conditions [8]. Paneth cells (as the essential intestinal stem-cell niche cells) constitute a major source of Wnt [9]. The unexpected phenotype observed in the irradiation model could therefore be explained by the fact that YAP deletion increases the number of Paneth cells upon (irradiation-induced or Rspo1-stimulated) intestinal regeneration (Figure 1). Further study of the role of YAP in driving Paneth cell differentiation may provide deeper insights into the Hippo pathway and tissue regeneration.

The Hippo signaling pathway is one of several pathways that play crucial roles in regulating the tissue regeneration. The current dogma states that the Hippo pathway is a growth/tumor-suppressive pathway, with its downstream effector Yki/YAP acting as a growth promoter/oncoprotein. The findings of Barry *et al.* [8] regarding YAP's functions in the intestine provide a layer of complexity to this simple view of YAP. In fact, both upregulation and downregulation of YAP expression have been reported in colorectal cancer patients [8,14]. For a better understanding of the role of YAP in colon cancer, it will be crucial to unravel the (likely opposing) direct and indirect effects of YAP on crypt stem cells and their niches. Crosstalk of the Hippo pathway with other signaling pathways in homeostatic control of crypts has been proposed. For example, TAZ and YAP have been reported to suppress the Wnt/β-catenin pathway through direct binding to Dishevelled [8,15] or β-catenin [16]. Further, Mst1/2 and YAP may regulate the Notch pathway through controlling expression of the Notch intracellular domain and Hes1 (see also above) [4,7]. Finally, YAP may activate the phosphatidylinositol 3-kinase and mTOR signaling pathways through suppression of the PTEN phosphatase [17]. However this story may unravel, the Hippo pathway is taking center stage as a key regulator of organ-size control, tissue regeneration and tumorigenesis.

References

1. Pan, D. (2010). The hippo signaling pathway in development and cancer. Dev. Cell *19*, 491–505.
2. Huang, J., Wu, S., Barrera, J., Matthews, K., and Pan, D. (2005). The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila homolog of YAP. Cell *122*, 421–434.
3. Dong, J., Feldmann, G., Huang, J., Wu, S., Zhang, N., Comerford, S.A., Gayyed, M.F., Anders, R.A., Maitra, A., and Pan, D. (2007). Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell *130*, 1120–1133.
4. Camargo, F.D., Gokhale, S., Johnnidis, J.B., Fu, D., Bell, G.W., Jaenisch, R., and Brummelkamp, T.R. (2007). YAP1 increases organ size and expands undifferentiated progenitor cells. Curr. Biol. *17*, 2054–2060.
5. Karpowicz, P., Perez, J., and Perrimon, N. (2010). The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. Development *137*, 4135–4145.
6. Cai, J., Zhang, N., Zheng, Y., de Wilde, R.F., Maitra, A., and Pan, D. (2010). The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program. Genes Dev. *24*, 2383–2388.
7. Zhou, D., Zhang, Y., Wu, H., Barry, E., Yin, Y., Lawrence, E., Dawson, D., Willis, J.E., Markowitz, S.D., Camargo, F.D., *et al.* (2011). Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance. Proc. Natl. Acad. Sci. USA *108*, E1312–E1320.
8. Barry, E.R., Morikawa, T., Butler, B.L., Shrestha, K., de la Rosa, R., Yan, K.S., Fuchs, C.S., Magness, S.T., Smits, R., Ogino, S., *et al.* (2012). Restriction of intestinal stem cell expansion and the regenerative response by YAP. Nature *493*, 106–110.
9. Sato, T., van Es, J.H., Snippert, H.J., Stange, D.E., Vries, R.G., van den Born, M., Barker, N., Shroyer, N.F., van de Wetering, M., and Clevers, H. (2011). Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature *469*, 415–418.
10. Carmon, K.S., Gong, X., Lin, Q., Thomas, A., and Liu, Q. (2011). R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. Proc. Natl. Acad. Sci. USA *108*, 11452–11457.
11. de Lau, W., Barker, N., Low, T.Y., Koo, B.K., Li, V.S., Teunissen, H., Kujala, P., Haegebarth, A., Peters, P.J., van de Wetering, M., *et al.* (2011). Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. Nature *476*, 293–297.
12. Kim, K.A., Kakitani, M., Zhao, J., Oshima, T., Tang, T., Binnerts, M., Liu, Y., Boyle, B., Park, E., Emtage, P., *et al.* (2005). Mitogenic influence of human R-spondin1 on the intestinal epithelium. Science *309*, 1256–1259.
13. Okamoto, R., Tsuchiya, K., Nemoto, Y., Akiyama, J., Nakamura, T., Kanai, T., and Watanabe, M. (2009). Requirement of Notch activation during regeneration of the intestinal epithelia. Am. J. Physiol. Gastrointest. Liver Physiol. *296*, G23–G35.
14. Konsavage, W.M., Jr., Kyler, S.L., Rennoll, S.A., Jin, G., and Yochum, G.S. (2012). Wnt/beta-catenin signaling regulates Yes-associated protein (YAP) gene expression in colorectal carcinoma cells. J. Biol. Chem. *287*, 11730–11739.
15. Varelas, X., Miller, B.W., Sopko, R., Song, S., Gregorieff, A., Fellouse, F.A., Sakuma, R., Pawson, T., Hunziker, W., McNeill, H., *et al.* (2010). The Hippo pathway regulates Wnt/beta-catenin signaling. Dev. Cell *18*, 579–591.
16. Imajo, M., Miyatake, K., Iimura, A., Miyamoto, A., and Nishida, E. (2012). A molecular mechanism that links Hippo signalling to the inhibition of Wnt/beta-catenin signalling. EMBO J. *31*, 1109–1122.
17. Tumaneng, K., Schlegelmilch, K., Russell, R.C., Yimlamai, D., Basnet, H., Mahadevan, N., Fitamant, J., Bardeesy, N., Camargo, F.D., and Guan, K.L. (2012). YAP mediates crosstalk between the Hippo and PI(3)K-TOR pathways by suppressing PTEN via miR-29. Nat. Cell Biol. *14*, 1322–1329.

[1]Hubrecht Institute – KNAW and University Medical Centre Utrecht, Uppsalalaan 8, 3584CT Utrecht, the Netherlands. [2]Current address: Division of Stem Cell Biology and Developmental Genetics, MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.
*E-mail: vli@nimr.mrc.ac.uk

http://dx.doi.org/10.1016/j.cub.2012.12.021

Paleoanthropology: Five's a Crowd in Our Family Tree

Two new fossil jawbones from Kenya are claimed to confirm a diversity of early *Homo* species. However, archaic species concepts and an inadequate fossil record continue to obscure the origins of our genus.

Tim White

Human bones are common in cemeteries, but remains of our more ancient ancestors and relatives are fewer, further between and notoriously difficult to recover. This is particularly true for fossils that are millions of years